Transmission of viruses by artificial breeding techniques: a review[1]

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Artificial insemination has been used extensively by animal breeders since the 1930s and 1940s, mainly in cattle and to some extent in pigs and sheep. It has not gained favour among pedigree horse breeders.

By using the techniques available, semen can be collected from male animals at an artificial insemination centre and stored for varying periods at low temperatures. The semen can then be distributed to the surrounding area and throughout the country, and can also be exported to other countries. Thus, as well as providing desired genetic characteristics, the semen could act as a vehicle for the wide distribution of undesirable pathogens. Consequently, it is necessary to be aware of those viruses likely to be present in the semen and of their ability to spread through artificial insemination.

The viruses that may be present can be considered in three categories: (1) contaminants from the environment during semen collection, e.g. enteroviruses; (2) those that are shed into the semen and spread in this way; and (3) those that may be present within the germ cell or spermatozoa. Included in category (2) are foot-and-mouth disease, bluetongue, bovine herpes 1, bovine leukosis, bovine diarrhoea, border disease, bovine ephemeral fever, lumpyskin disease and paravaccinia viruses. In certain circumstances bluetongue, bovine diarrhoea and border disease viruses may be regarded as belonging to category (3). The viruses that may be present in bovine serum, and vertical transmission of viruses, have been reviewed by Kahrs *et al.* (1980) and Mims (1981), respectively.

In this paper foot-and-mouth disease, bluetongue and bovine herpes 1 viruses are discussed in terms of the amounts of virus present in the semen of infected and vaccinated males, the amounts of virus required to infect the female by artificial insemination, and the factors such as the epidemiological picture in the country and the infective status of the donor which may influence the likelihood of infection being present.

Foot-and-mouth disease

Foot-and-mouth disease is a highly contagious disease of cattle, sheep, goats and pigs, and is characterized by fever and development of vesicular lesions around and in the mouth and on the feet. It is caused by an aphthovirus (family Picornaviridae).

Foot-and-mouth disease virus (titres of $10^{1.5}$–$10^{4.7}$ mouse LD_{50} per ml) has been found in bull semen up to four days before clinical signs of the disease. When lesions appeared, the semen contained $10^{6.2}$ mouse LD_{50} per ml, and virus ($10^{1.4}$ mouse LD_{50} per ml) was found five days later. Trace amounts of virus were present in the semen for a further 37 days. Affected bulls were reluctant to serve and semen had to be collected by electro-ejaculator. The semen was of low quality (Cottral *et al.* 1968, Sellers *et al.* 1968).

Boars also excrete virus into the semen before and during the appearance of disease. The amounts of virus present were low (McVicar *et al.* 1977).

The source of virus in the semen is due to generalization of virus from the original site of infection via the lymphatics and blood stream to the testis and accessory glands. Virus may also multiply in the skin around the preputial orifice.

The foot-and-mouth disease vaccines that are available inhibit generalization of virus but

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do not necessarily protect the primary sites of infection in the pharynx or on the skin. No virus has been found in the semen of vaccinated bulls, but trace amounts of virus were present on the prepuce and on the coat of the animal and could therefore contaminate the semen (Sellers *et al.* 1969, Sellers *et al.* 1977). Virus has been isolated from the semen of a vaccinated bull that had been exposed to infection and developed benign lesions (Callis *et al.* 1976).

The minimum amount of virus to infect cows or heifers by insemination is $10^{3.4}$ MLD_{50} (Cottral *et al.* 1968); sows and goats, however, do not appear to be infected by this route.

Semen would not be collected during clinical disease. Thus, the main danger of spread comes from semen collected from bulls before the onset of clinical disease, from the semen of partially-protected bulls showing benign lesions and from contamination of semen of vaccinated bulls in contact with disease. Since semen is stored after collection, observations on the donors can be made until all likelihood of disease of virus developing can be excluded. The disease situation on the farm and in the area can be assessed. Tests to determine if the animal is or has been infected can be carried out; in addition, an aliquot of the semen can be tested for freedom from virus.

Bluetongue

Bluetongue is a non-contagious disease of ruminants characterized by congestion, oedema and haemorrhage in the infected animal. The disease is caused by an orbivirus (Reoviridae) which is transmitted biologically by Culicoides midges. Sheep are the species mainly affected. In cattle there may be some disease, but the majority of infection is silent although the virus multiplies in cattle. In pregnant cows or heifers infected for the first time, the virus may cause abortions, fetal malformations or birth of calves with arthrogryposis or hydranencephaly.

In two of eight bulls infected for the first time, bluetongue virus was found in the semen nine days after infection. Titres of $10^{5.7}$ ID_{50} per ml at 15 days, and $10^{4.1}$ ID_{50} per ml at 28 days, were found. Virus was detectable at 35 days (Howard 1982). Other workers have found virus in the semen from 7 to 106 days after infection (Luedke *et al.* 1975).

In cattle infected with bluetongue, virus can be isolated from blood cells, erythrocytes, leukocytes and monocytes for about 110 days. Virus has also been found in macrophages, in the endothelial cells lining blood vessels and in cells of the reticular endothelial system. In bulls infected in the post-pubetal period the virus is believed to be present in the semen as a result of infiltration of infected blood cells into the genitalia (Howard 1982).

The amount of virus in semen required to infect heifers or cows is about $10^{1.25}$ ID_{50} (R A Bowen 1982, personal communication). Thus semen collected from infected bulls over a period of about 25 days could be infectious for heifers. The heifer will become infected, showing viraemia and developing antibodies. If ova are fertilized, bluetongue virus has no effect on them while the zona pellucida is intact (Bowen *et al.* 1982, Sing *et al.* 1982).

Bluetongue is found in areas north and south of the equator, as far north as the USA/Canada border, the Mediterranean, Turkey, Iran, Pakistan, India, China and Japan, and as far south as Paraguay, South Africa and south-east Australia (Sellers 1981). At lower latitudes and altitudes it is endemic but elsewhere it is epidemic or sporadic. The effects on import and export have been reviewed by Sellers & Taylor (1980). In epidemic and sporadic areas the virus is introduced by movement of infected animals or by Culicoides midges carried on the wind. Thus, mature bulls may have escaped infection while young. Such an incursion by animals or midges may be detected by serological tests carried out on sentinel herds every year. In addition, serological tests can be carried out on the bulls in the artificial insemination centres. Where evidence of infection is detected in the areas liable to incursions, or where evidence of infection is found in bulls, the use or import of semen from that area or centre can be avoided. As a further preventive measure, collection of semen can be carried out during the winter when, owing to adverse conditions, the Culicoides vector is not active. Finally, further tests can be done on portions of semen for freedom from bluetongue virus.

Bovine herpes virus 1

Bovine herpes virus 1 has been associated with respiratory, ocular, reproductive, central nervous system and other systemic infections of cattle – that associated with the respiratory and ocular infections has been termed infectious bovine rhinotracheitis virus, and that with reproductive infections, infectious pustular vulvovaginitis and infectious balanoposthitis. Infected cows and heifers develop pustules and ulcers in the vulva which heal but a purulent discharge may be found. Similar lesions develop on the mucosa of the penis and prepuce of bulls. The virus is transmitted at natural service or in the semen at artificial insemination or by fomites (Gibbs & Rweyemamu 1977).

In primary experimental infection of bulls, virus can be found in the preputial washings for 5 to 30 days, with titres of 10^5–10^6 $TCID_{50}$ per ml (Bitsch 1973). Thereafter, intermittent excretion of virus in the preputial washings occurred for 840 days, with titres of up to $10^{3.5}$ ID_{50} per ml being found. In other work virus has been isolated from preputial washings for 361 days (Snowdon 1965). Excretion of virus can be stimulated by the administration of corticosteroids. It is not clear whether further lesions are found at the time of intermittent virus excretion but, after the administration of corticosteroids, local lesions were found. It would thus appear that once a bull has been infected it is liable to excrete virus intermittently for the remainder of its life. Field outbreaks have been described by Huck *et al.* (1971) and Collings *et al.* (1972). The virus that is found in the semen is derived from the mucosa of the penis and prepuce rather than from a viraemia.

Live vaccines have been produced against the respiratory form, infectious bovine rhinotracheitis, and are said to protect against infectious pustular vulvovaginitis and infectious balanoposthitis (*see* Kahrs *et al.* 1980).

The amounts of virus required to infect cows or heifers by the intravaginal route appear to be low – of the order of 10 ID_{50}. Infection of semen with bovine herpes virus 1 may or may not lead to infertility.

Attempts to get rid of the virus once it has established itself are generally unsuccessful. The aim, therefore, is to avoid infection reaching artificial insemination centres by disposing beforehand of those bulls that have antibodies or excrete virus. Aliquots of the semen can be tested for freedom from virus. However, there can be problems in maintaining freedom in an artificial insemination centre.

Equid herpes virus 3

Artificial insemination is not used for horses. However, the equid herpes virus 3 gives rise to a disease similar to bovine herpes 1, known as equine coital exanthema. The lesions are seen as vesicles, pustules or ulcers on the vulva and penis and on the udder of mares and lips of suckling foals. Recurrence of lesions is common and spread is by coitus or from dam to young or by fomites (Bryans 1980).

Virus is excreted intermittently and once present in a horse stud may recur frequently, with or without the appearance of clinical signs. An outbreak in a closed pony herd has been described by Burrows & Goodridge (1978).

The disease and intermittent excretion of virus with bovine herpes 1 and equid herpes 3 resemble closely human genital herpes.

Viruses that may be transmitted in germ cells

With foot-and-mouth disease, bluetongue in mature bulls and bovine herpes 1, virus is present in the semen through local lesions on preputial or penile mucosae or introduction through blood cellular elements. The same is found with bovine leukosis virus, in which infected lymphocytes are present in the semen. Where animals are infected *in utero* or possibly before puberty, virus may be present in the gonocytes or in the spermatozoa. Specific fluorescence in the gonocytes has been described for border disease (Gardner 1980). Abnormalities and virus-like particles have been described in spermatozoa from bulls latently infected with bluetongue virus (Foster *et al.* 1980).

Conclusions

There are technical problems in attempting to isolate viruses from semen. These have been detailed by Kahrs *et al.* (1980) and include toxicity of semen for cell cultures, unweaned mice and fertile eggs, loss of virus during preparation and antiviral activity of some semen. In this paper the aim has been to demonstrate how knowledge of the time of excretion of virus in the semen, the dose required to infect by artificial insemination, the behaviour of the virus in the animal, the disease status of the area and of the animal itself can exclude unsuitable areas, artificial insemination centres or animals so that the minimum of semen needs to be tested. This is of some importance where semen from valuable bulls is concerned.

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